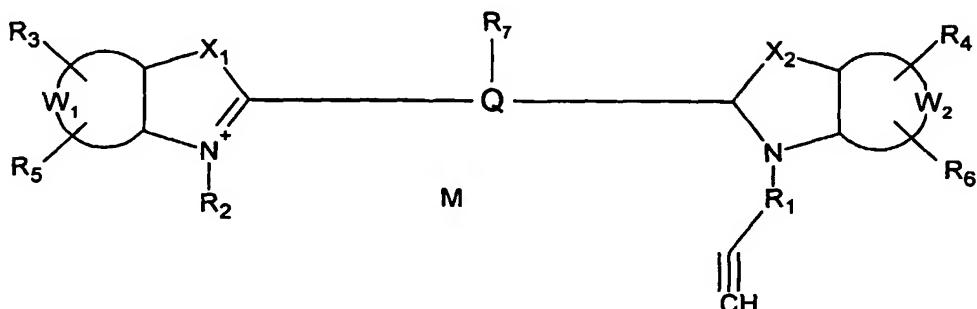


CLAIMS

1. A cyanine modified with an alkynyl-linker arm, having the following general formula (I), including the valence tautomers thereof:



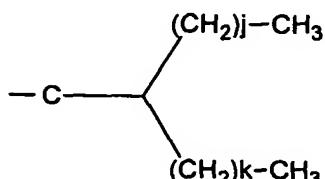
5

(I)

wherein

R₁ is a linear, saturated or unsaturated alkyl chain, having from 1 to 30 carbon atoms, wherein one or more carbon atoms are each optionally substituted by a component independently selected from an oxygen or a sulfur atoms, a -NH- or a -CONH- group, or a cyclic 4-, 5- or 6- membered grouping of carbon atoms, aromatic or not aromatic, wherein one or more carbon atoms are each optionally substituted by a heteroatom independently selected from oxygen, sulfur, nitrogen and selenium; W₁ and W₂ are independently selected from a benzene ring and a naphthalene ring wherein one or more carbon atoms are optionally substituted by one or more heteroatoms selected from oxygen, sulfur, selenium and nitrogen, or one of W₁ and W₂ is absent, or both of them are absent; X₁ and X₂ are independently selected from the group consisting of -O-, -S-, -Se-, -N-, -C(CH₃)₂, -CH=CH-, -NH-, and

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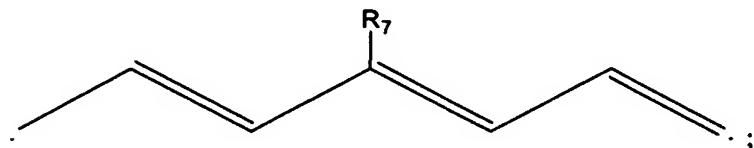
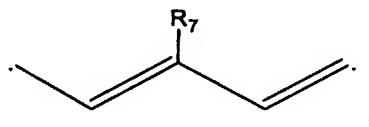
with j = 1-20 and k = 1-20;

R₂, R₃, R₄, R₅ and R₆ are independently selected from hydrogen, -COOH, -OH, -NO₂, -OCH₃, -SO₃H, -SO₃⁻, and -R₈-Y wherein R₈ is a linear, saturated or unsaturated alkyl chain, having from 1 to 30 carbon atoms, wherein one or more carbon atoms are each optionally substituted by a component independently

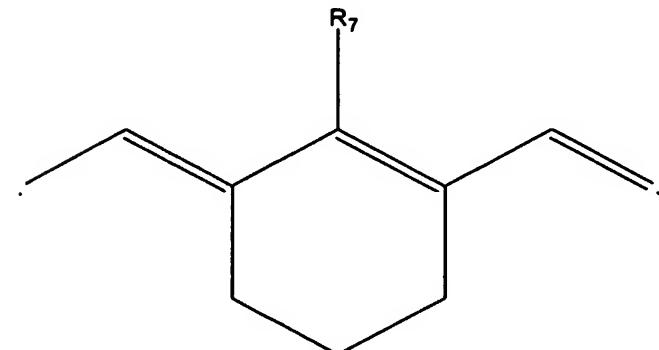
selected by an oxygen or a sulfur atom, a $-\text{NH}-$ or a $-\text{CONH}-$ group, or a cyclic 4-, 5- or 6- membered grouping of carbon atoms, aromatic or not aromatic, wherein one or more carbon atoms are each optionally substituted by a heteroatom independently selected from oxygen, sulfur, nitrogen or selenium, and wherein Y is selected from the group consisting of hydrogen, carboxyl, carbonyl, amino, sulphydryl, thiocyanate, isothiocyanate, isocyanate, maleimide, hydroxyl, phosphoramidite, glycidyl, imidazolyl, carbamoyl, anhydride, bromoacetamido, chloroacetamido, iodoacetamido, sulphonyl halide, acyl halide, aryl halide, hydrazide, succinimidyl ester, hydroxysulfosuccinimidyl ester, phthalimidyl ester, naphthalimidyl ester, monochlorotriazine, dichlorotriazine, mono- or di- halide substituted pyridine, mono- or di- halide substituted diazine, aziridine, imidic ester, hydrazine, azidonitrophenyl, azide, 3-(2-pyridylthio)-propionamide, glyoxal, aldehyde, nitrophenyl, dinitrophenyl, trinitrophenyl and $-\text{C}\equiv\text{CH}$;

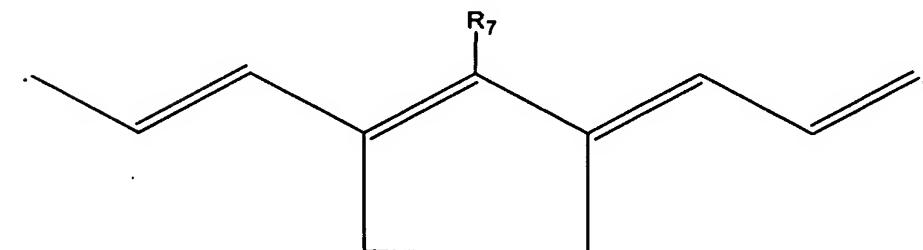
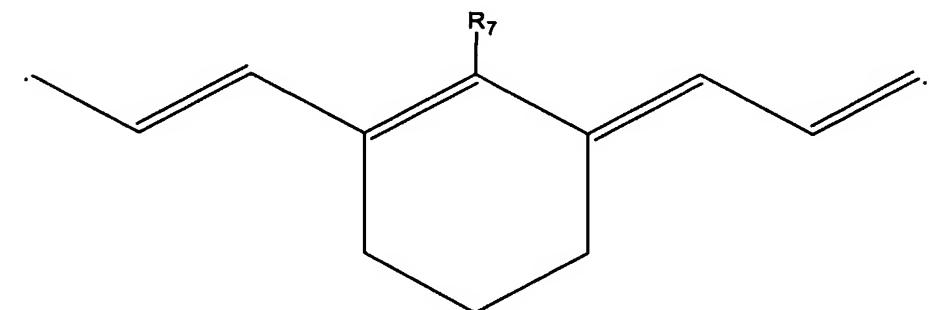
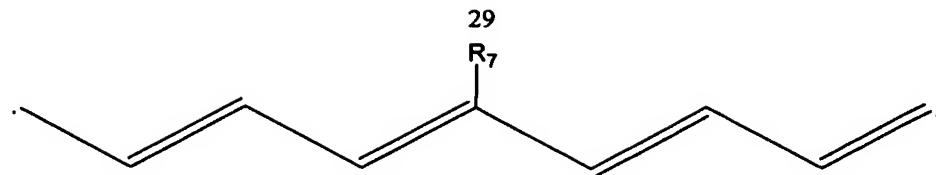
M is a counterion; and

15 Q is a polymethinic chain selected from:



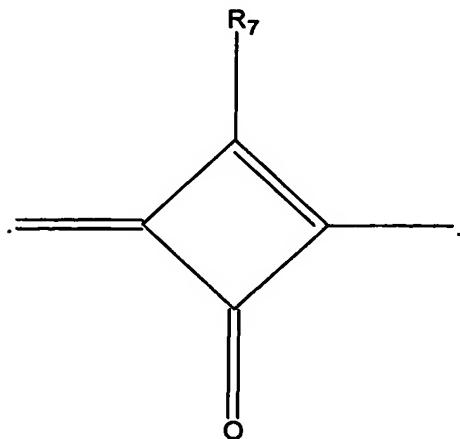
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5

or



wherein R_7 is selected from the group consisting of hydrogen, fluorine, chlorine, bromine, iodine, phenoxy, thiophenoxy, anilino, cyclohexylamino, piridine, $-R_8-Y$, $-O-R_8-Y$, $-S-R_8-Y$, $-NH-R_8-Y$, wherein R_8 and Y are as defined above, and aryl optionally substituted by one or more substituents independently selected from the group consisting of $-SO_3H$, carboxyl ($-COOH$), amino ($-NH_2$), carbonyl ($-$

10

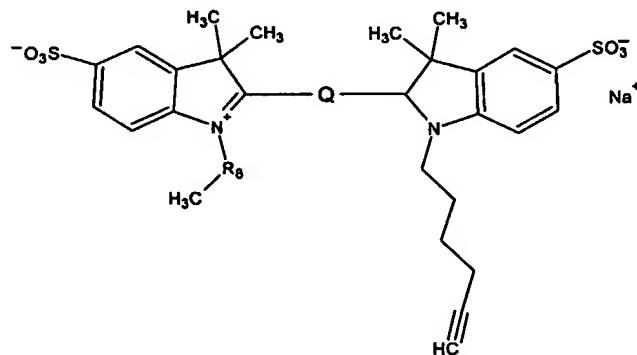
CHO), thiocyanate (—SCN), isothiocyanate (—CNS), epoxy and —COZ wherein Z represents a leaving group.

2. The cyanine according to claim 1, wherein said leaving group is selected from the group consisting of —Cl; —Br; —I; —OH; —OR₁₁; —OCOR₁₁, wherein R₁₁ is linear or branched alkyl having from 1 to 4 carbon atoms;

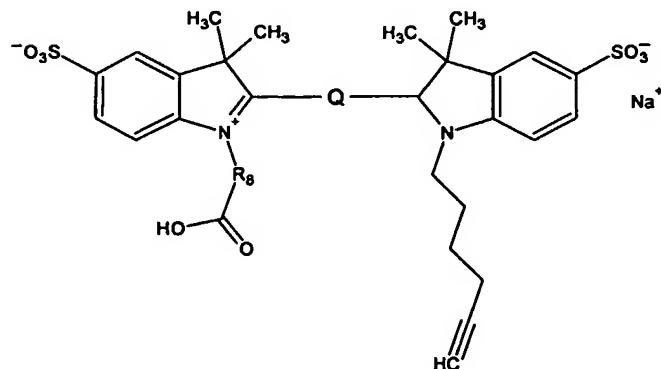
—O—CO—Ar, wherein Ar is aryl optionally substituted; —O—CO—Het, wherein Het is selected from succinimide, sulfosuccinimide, phthalimide and naphthalimide; —NR₂₂R₃₃, wherein R₂₂ and R₃₃ are each independently linear or branched alkyl having from 1 to 10 carbon atoms.

3. The cyanine according to claim 1 or 2, wherein one of R₂, R₃, R₄, R₅ and R₆ is —R₈—Y, wherein Y is different from H and from —C≡CH.

4. The cyanine according to claim 3 selected from the group consisting of:

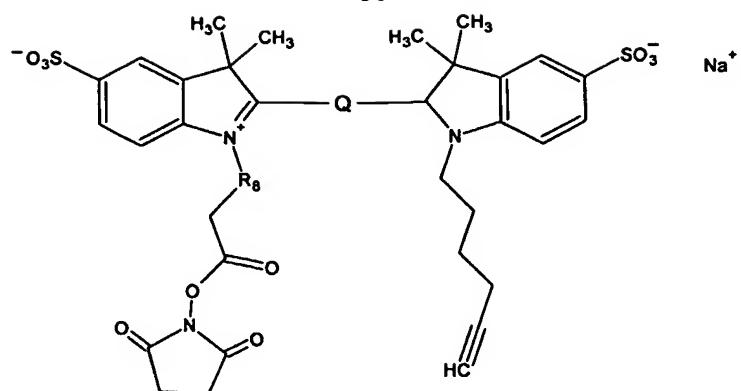


Formula (Ia)

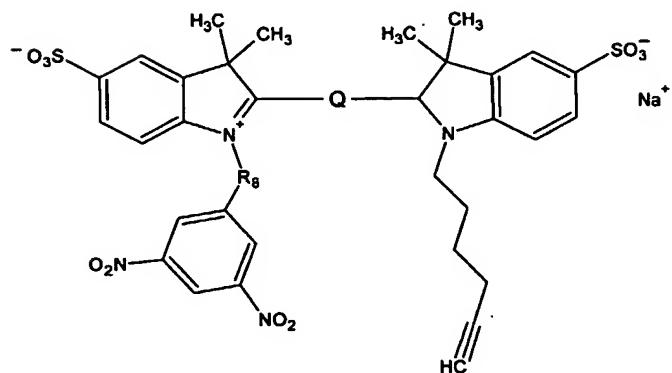


Formula (Ib)

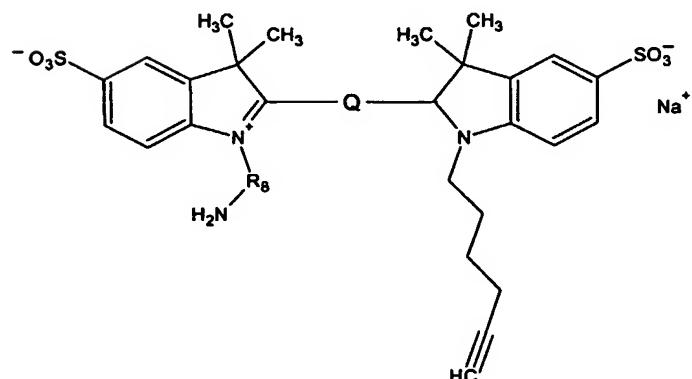
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Formula (Ic)

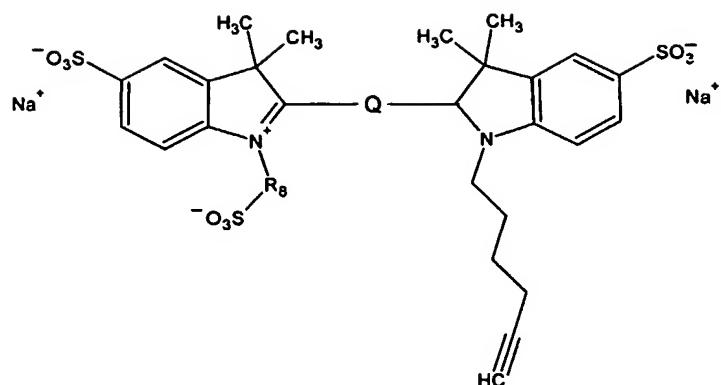


Formula (Id)

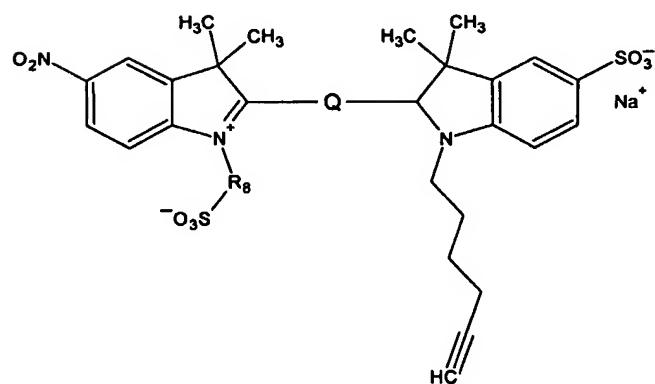


Formula (Ie)

32

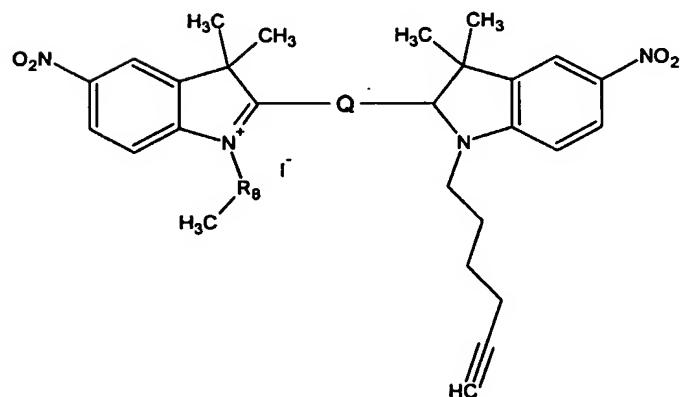


Formula (If)



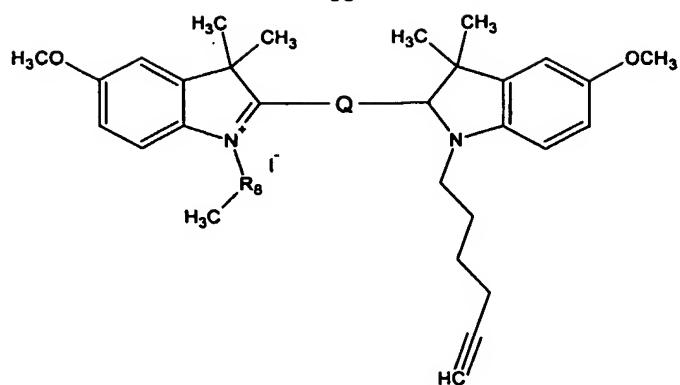
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Formula (Ig)

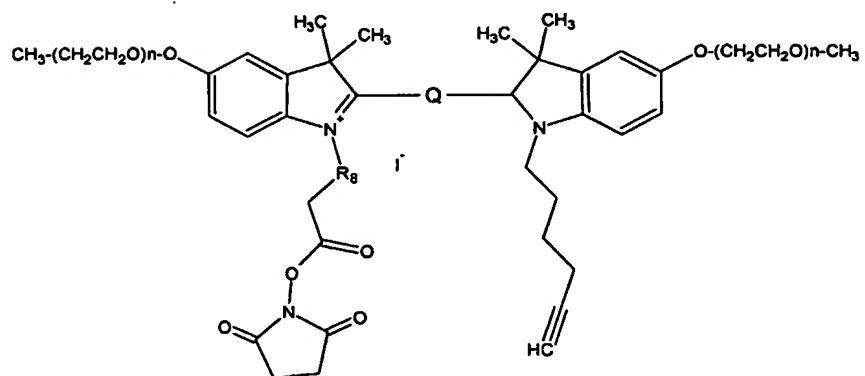


Formula (Ih)

33

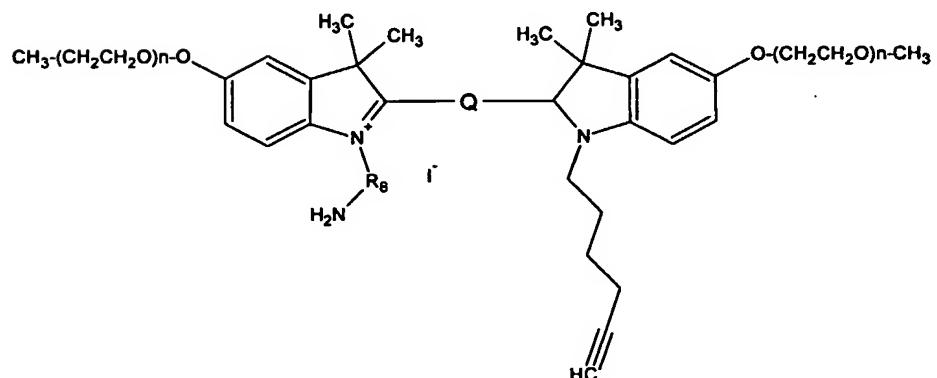


Formula (Ii)



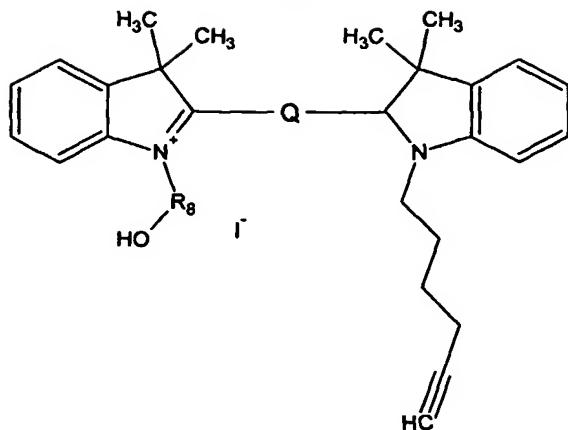
Formula (Ii)

5



Formula (Im)

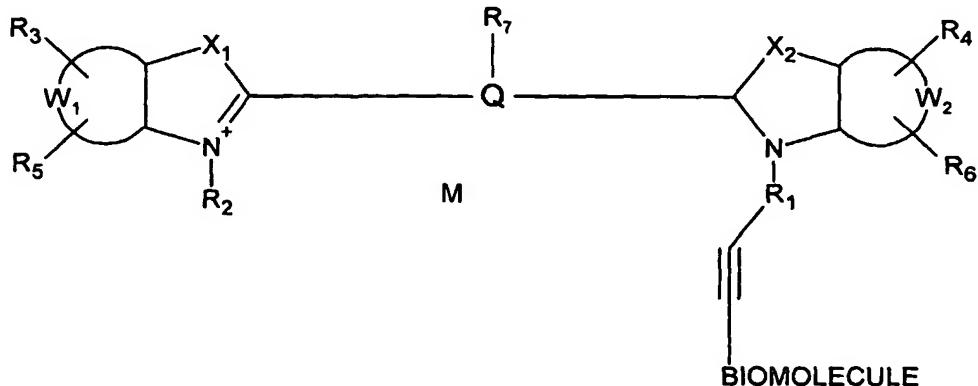
34



Formula (In),

wherein Q and R₈ are as defined in claim 1 and n is an integer between 1 and 100.

5. The cyanine according to any of the claims 1 to 4, conjugated through the linker arm -R₁-C≡CH with a biomolecule, said conjugated cyanine being represented by the general formula (II), including the valence tautomers thereof:

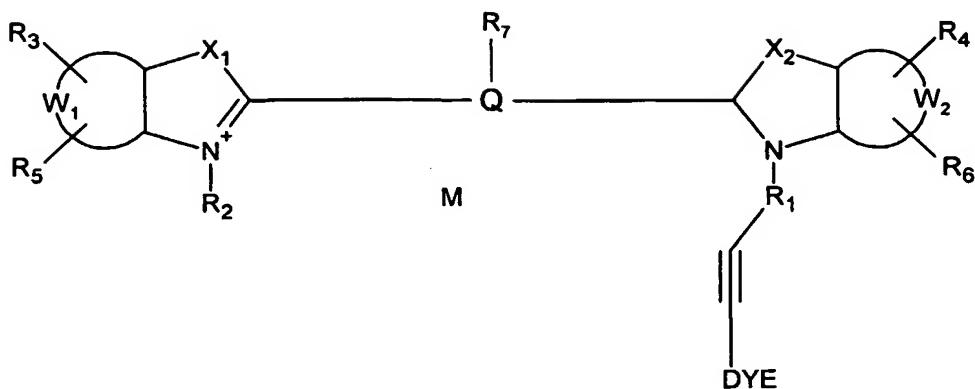


10. wherein R₁, R₂, R₃, R₄, R₅, R₆, R₇, W₁, W₂, X₁, X₂, Q and M are as defined in claim 1.

6. The cyanine according to claim 5, wherein said biomolecule is selected from the group consisting of nucleotides, nucleosides, oligonucleotides, nucleic acids, peptides and proteins.

15. 7. The cyanine according to any of the claims 1 to 4, conjugated through the linker arm -R₁-C≡CH with a second fluorescent dye, said second fluorescent dye being capable of emitting fluorescence at wavelengths at which the cyanine is capable of absorbing, or said fluorescent dye being capable of absorbing at wavelengths at which the cyanine is capable of emitting, said cyanine conjugated with a second fluorescent dye being represented by the general formula (III), including the valence tautomers thereof:

35



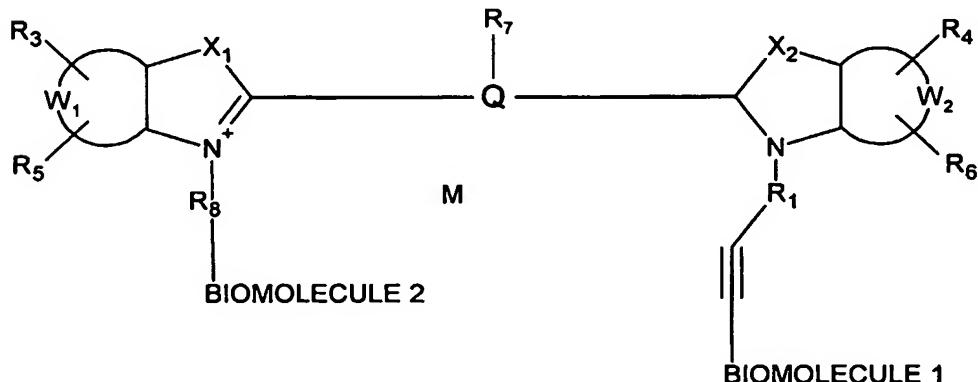
(III)

wherein R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , W_1 , W_2 , X_1 , X_2 , Q and M are as defined in claim 1.

5 8. The conjugated cyanine according to claim 7, wherein said second fluorescent dye is N,N' -Difluoroboryl-1,9-dimethyl-5-(4-iodophenyl)-dipyrromethane.

9. The conjugated cyanine according to claim 7, wherein said second fluorescent dye is a transition metal complex with at least one heterocyclic nitrogen-containing ligand.

10 10. The cyanine according to claim 3, conjugated through the linker arm $-R_1-C\equiv CH$ with a first biomolecule selected from the group consisting of nucleotides, nucleosides, oligonucleotides, nucleic acids, peptides, proteins, vitamins and hormones, and through the linker arm $-R_8-Y$ with a second equal or different biomolecule selected from the group consisting of nucleotides, nucleosides, oligonucleotides, nucleic acids, peptides, proteins, vitamins and hormones, said cyanine conjugated with a first and a second biomolecule being represented by the general formula (IV):



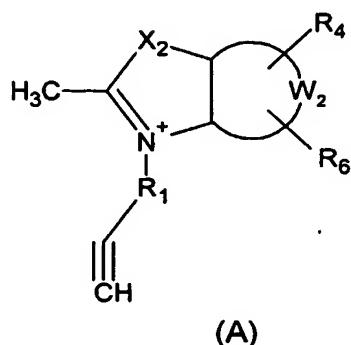
(IV)

20 wherein R_1 , R_3 , R_4 , R_5 , R_6 , R_7 , R_8 , W_1 , W_2 , X_1 , X_2 , Q and M are as defined in

claim 1.

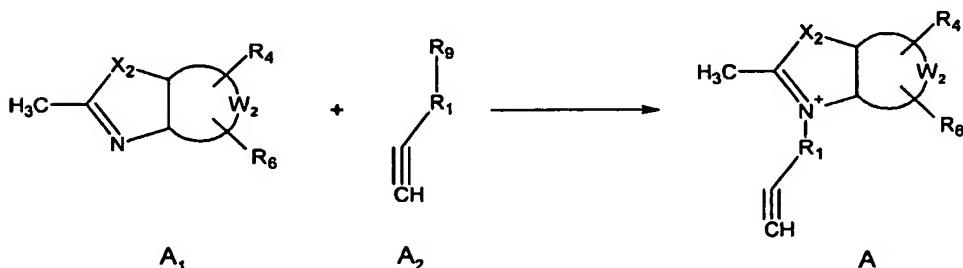
11. Intermediate for preparing a cyanine modified with an alkynyl linker arm of formula (I) as defined in claim 1, said intermediate being represented by the general formula (A):

5



wherein R₁, R₄, R₆, X₂, W₂ are as defined in claim 1.

12. A method for preparing an intermediate of formula (A) as defined in claim 11, comprising the step of reacting a nitrogen containing heterocyclic system of formula A₁ with a molecule containing a triple bond of formula A₂ to form a quaternary ammonium salt of formula A:



15. wherein X₂, R₁, R₄, R₆ and W₂ are as defined in claim 1, and R₉ is selected in the group consisting of iodine, chlorine, bromine, OH, sulfate and tosylate.

13. The use of a cyanine according to any of the claims 1 to 4 as a fluorescent marker for biomolecules or as a quencher.